

FIG.1B

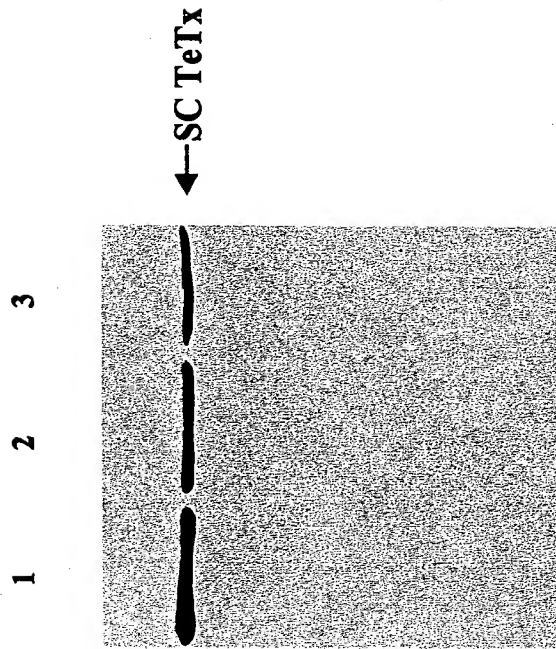


FIG.2B

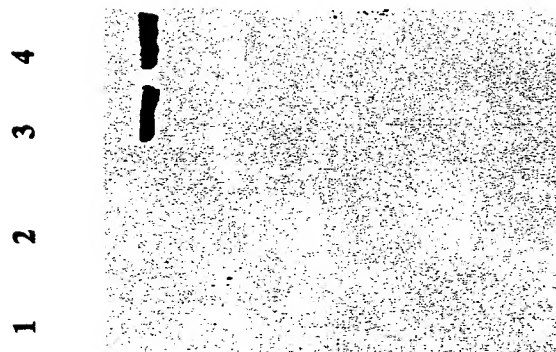


FIG.2A

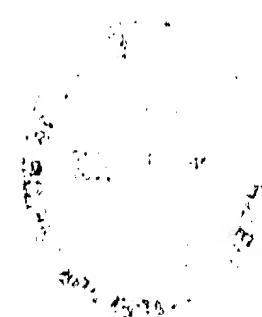
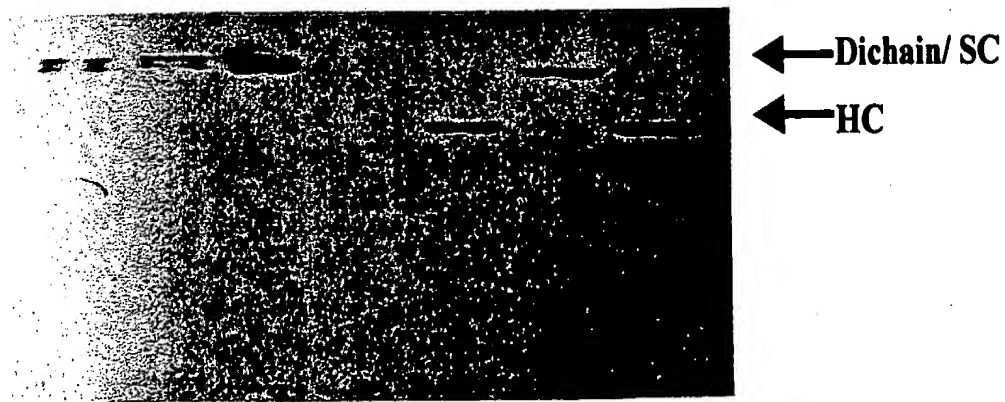
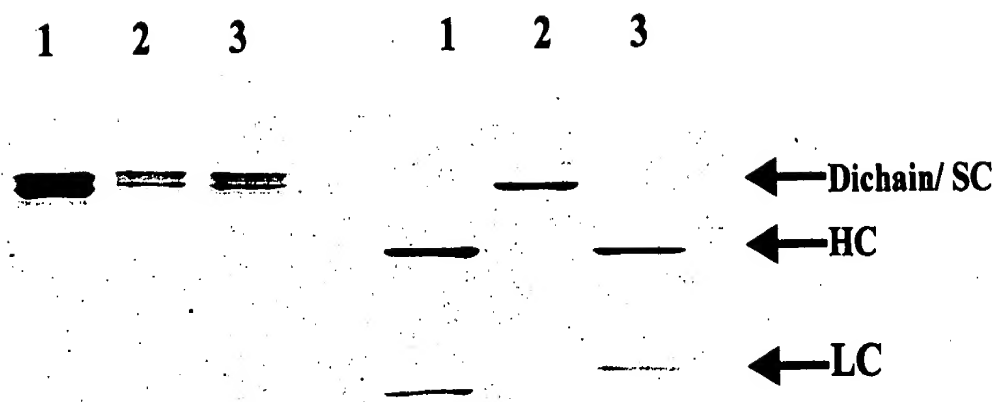


FIG.3A



Before reduction

After reduction

FIG 3B

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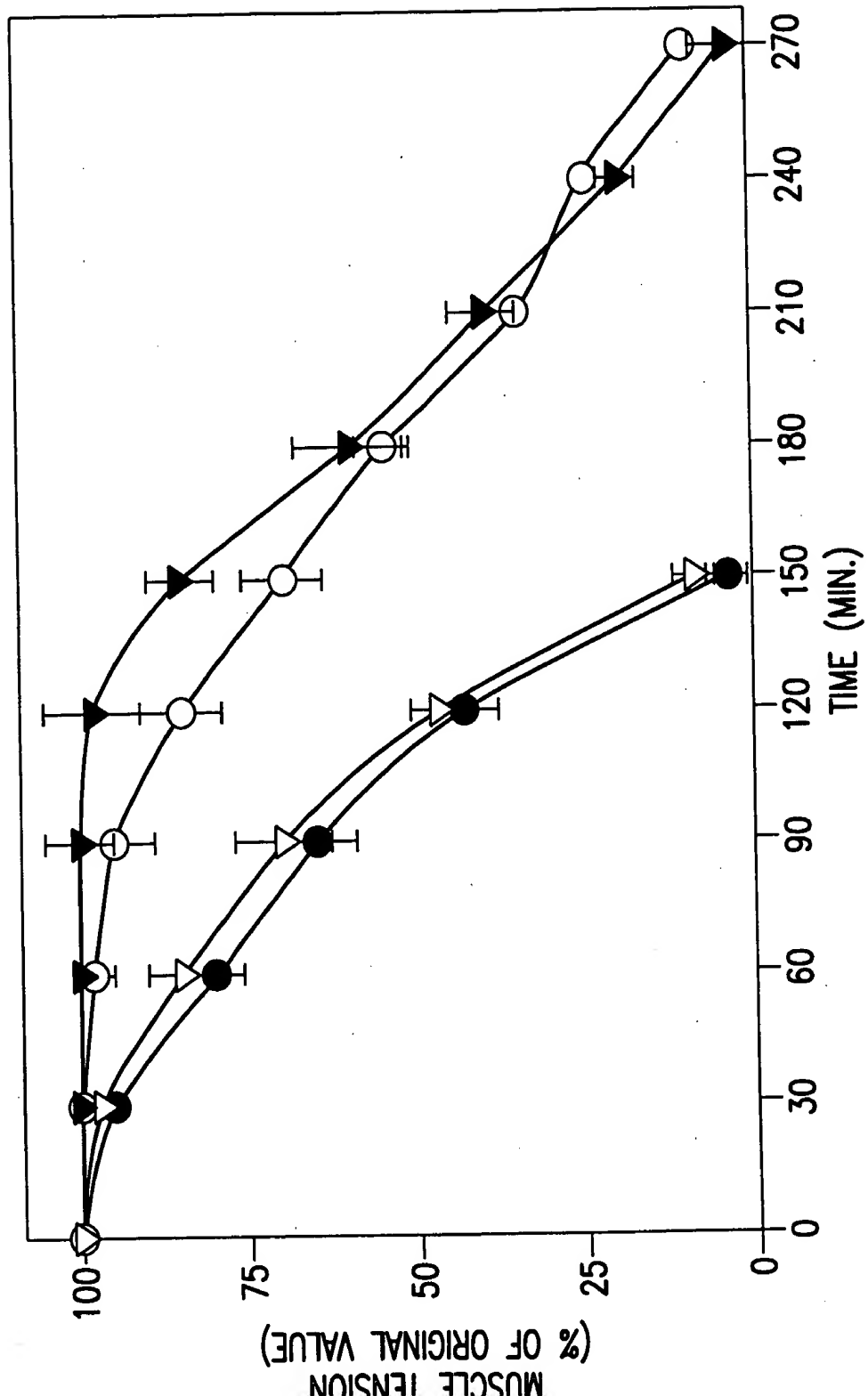


FIG. 4

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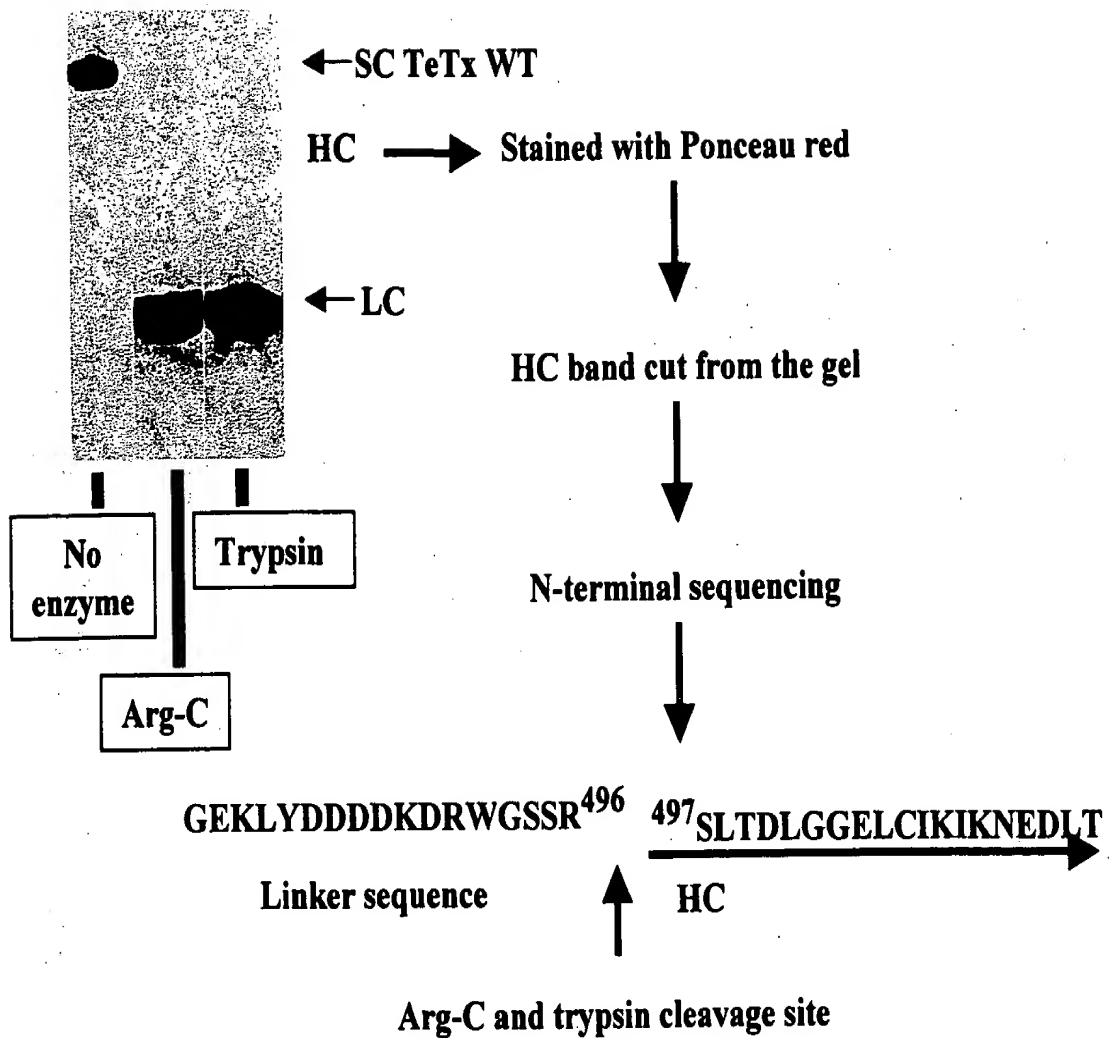


FIG 5

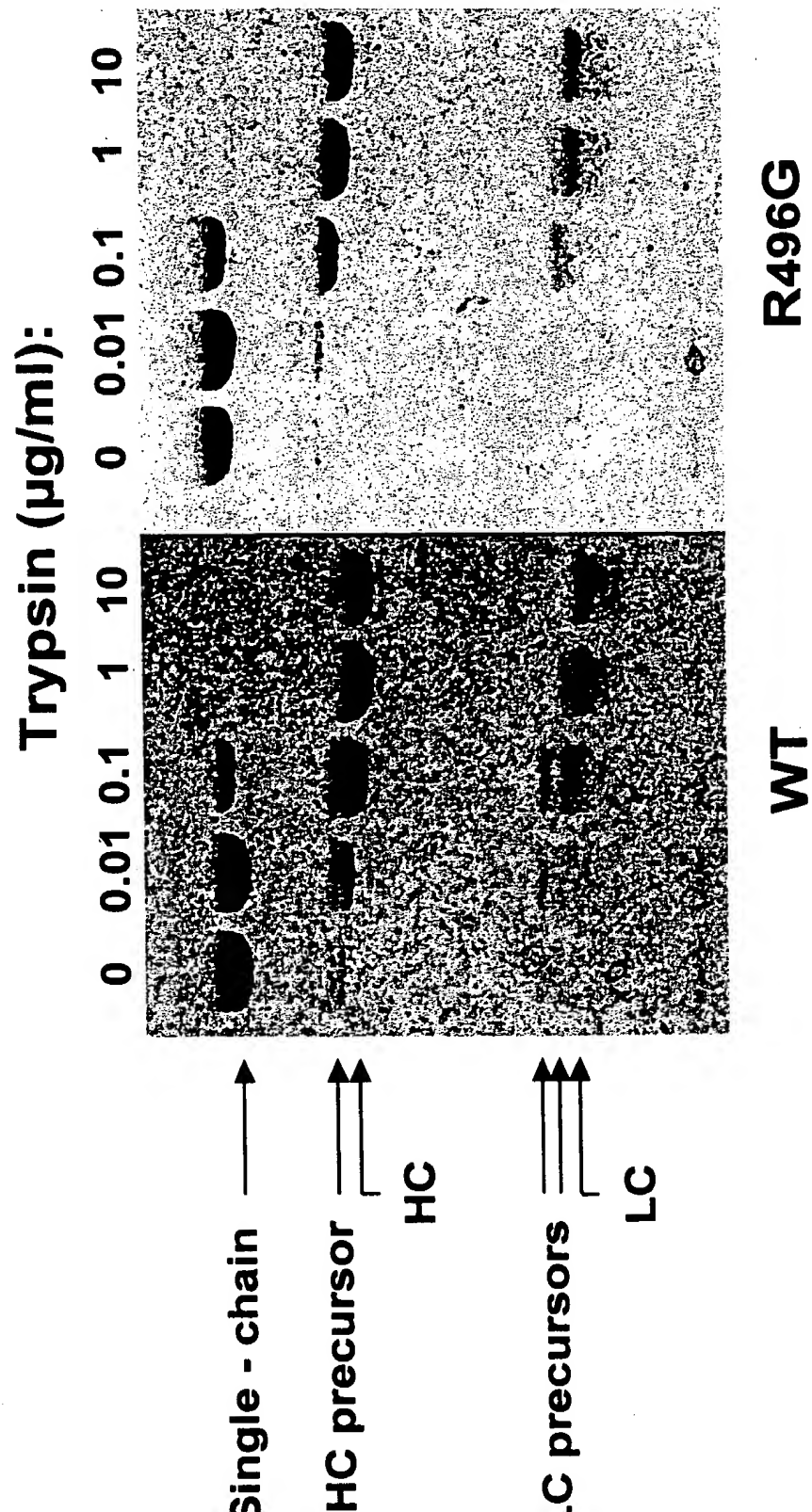


FIG.6

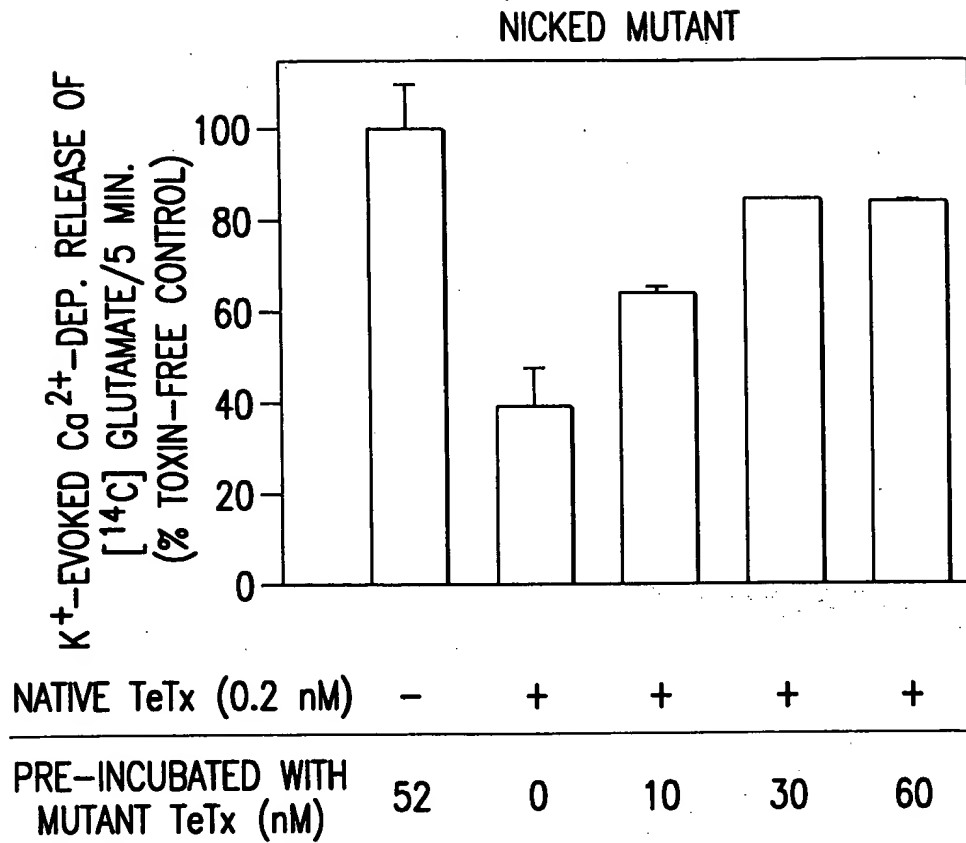


FIG.7A

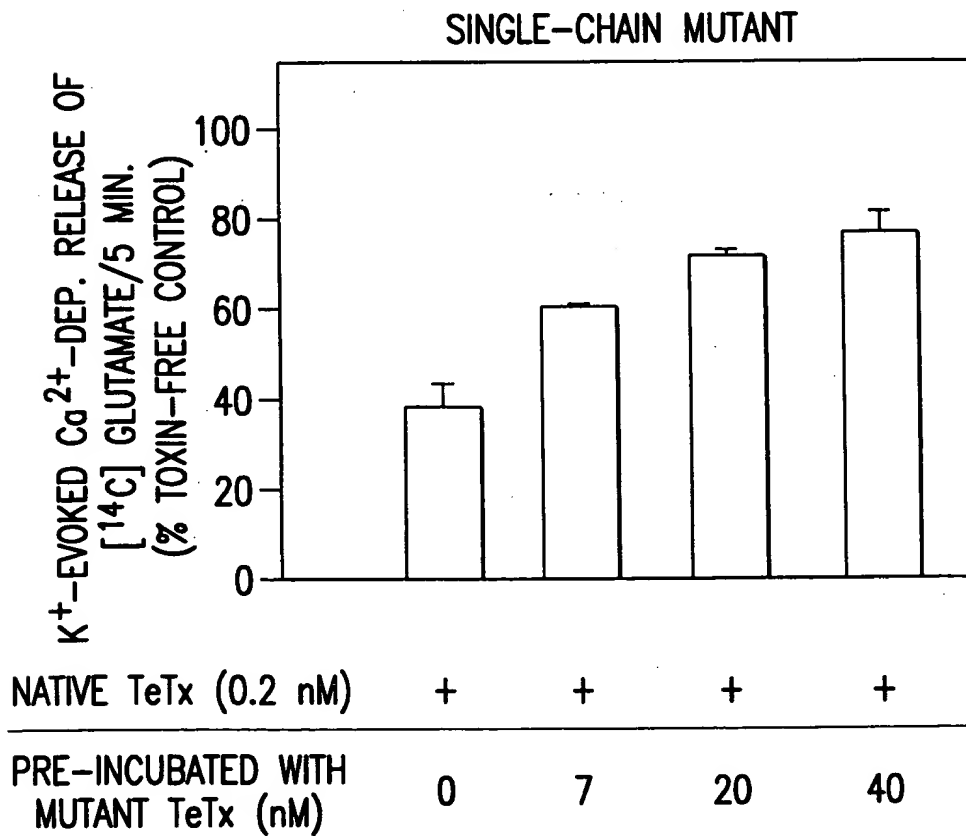


FIG 7B

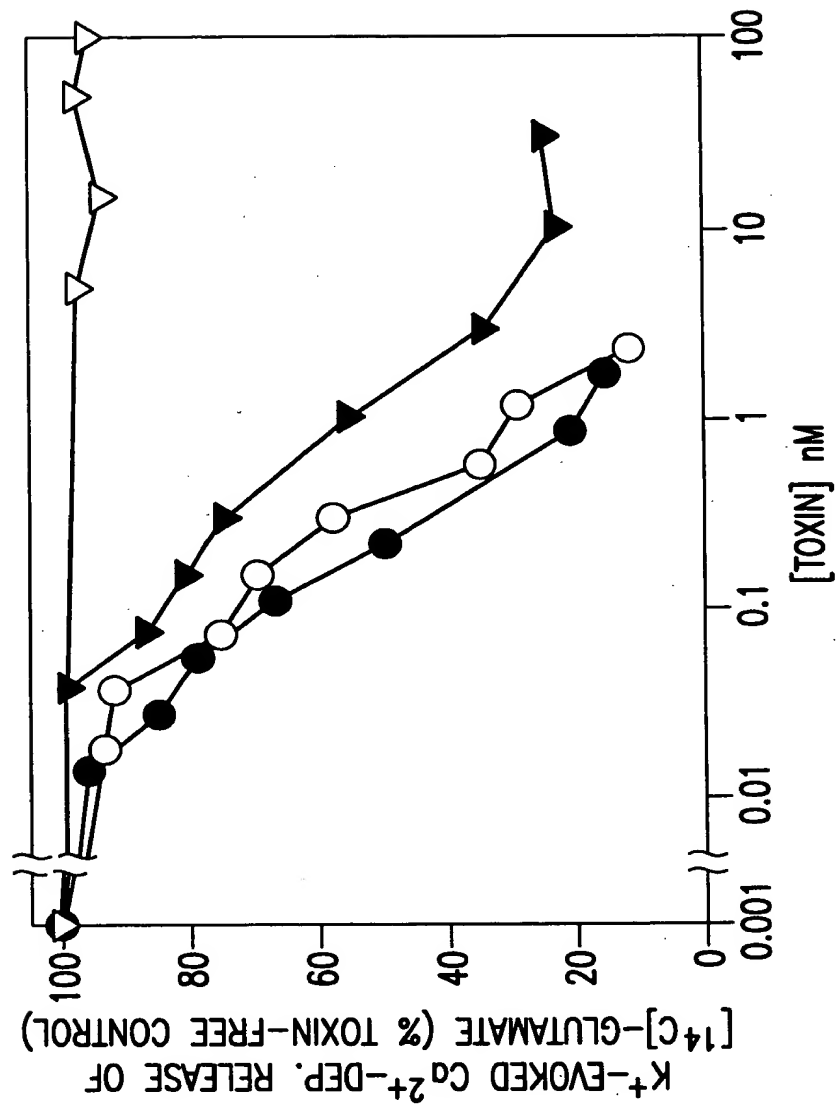


FIG.8

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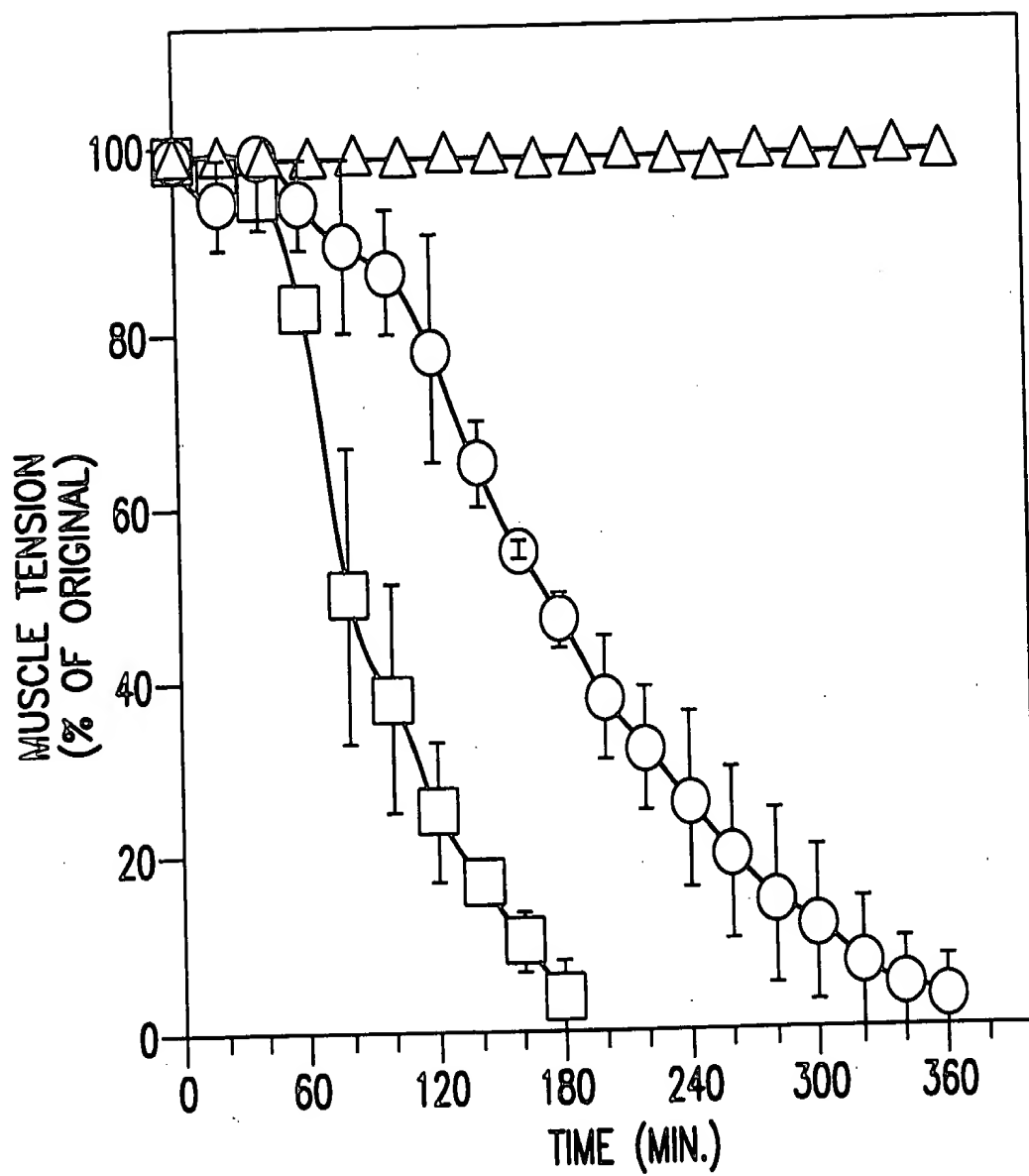
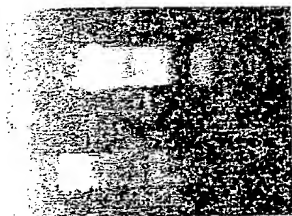


FIG 9

**CLOSTRIDIUM BOTULINUM TYPE E
(STRAIN BELUGA) CHROMOSOMAL DNA**

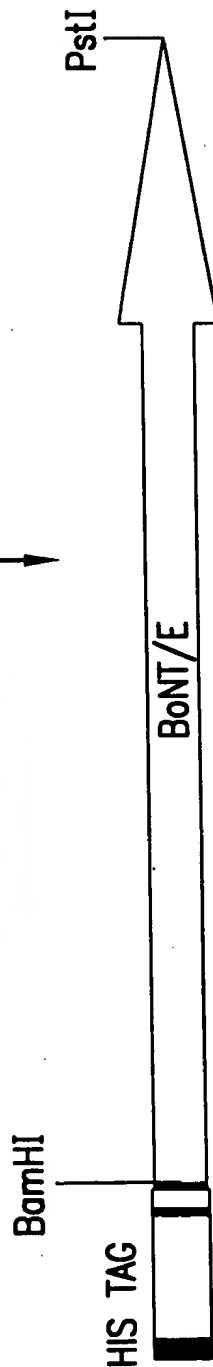
PCR WITH A PROOF READING
THERMOPOLYMERASE

1 2 3



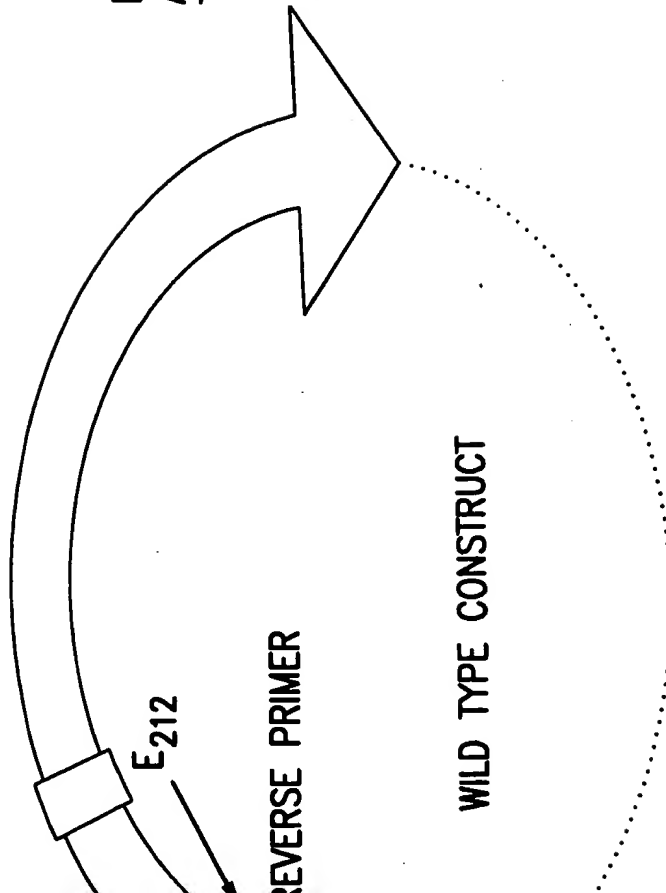
- 1: MARKERS
- 2: NEGATIVE CONTROL
- 3: SAMPLE

3.5-kB DNA FRAGMENT PURIFIED
AND CLONED INTO BamHI AND PstI
SITES OF pQE30 EXPRESSION VECTOR

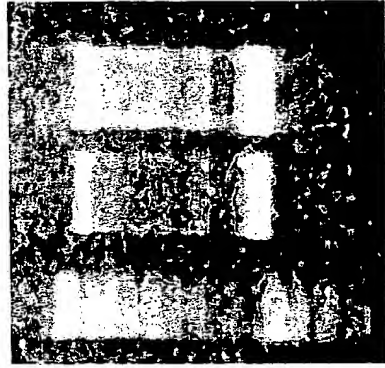


ATG	AGA	GGA	TCG	CAT	CAC	CAT	CAC	CAT	CAC	GGA	TCC	CCA	AAA	ATT	AAT	AGT	TTT
M	R	G	S	H	H	H	H	H	H	G	S	P	K	I	N	S	T
START					POLY HISTIDINE TAG					Bam HI		BoNT/E	LIGHT CHAIN				

FORWARD PRIMER WITH E212Q SUBSTITUTION



INVERSE PCR WITH
A PROOF READING
THERMOPOLYMERASE



7-kb DNA FRAGMENT PURIFIED,
LIGATED AND TRANSFORMED IN *E.COLI*

E212Q SINGLE-CHAIN MUTANT

FIG.11

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CULTURE OF E.COLI M15[pQEESCwt]



OVERNIGHT INDUCTION WITH IPTG



HARVEST, EXTRACTION AND CENTRIFUGATION

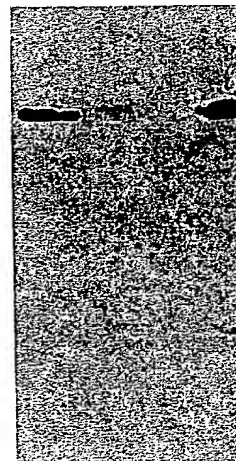
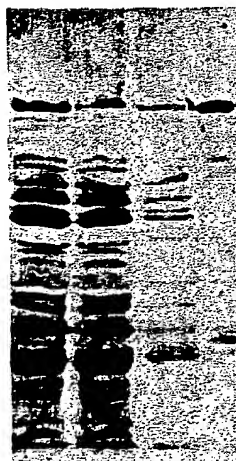


METAL CHELATE AFFINITY CHROMATOGRAPHY

SDS-PAGE (REDUCING COND.)

1 2 3 4 1 2 3 4

1: CELL-FREE EXTRACT
2: FLOW-THROUGH
3: WASH
4: ELUATE
(0.15M IMIDAZOLE)



← 150 kDa
BoNT SC

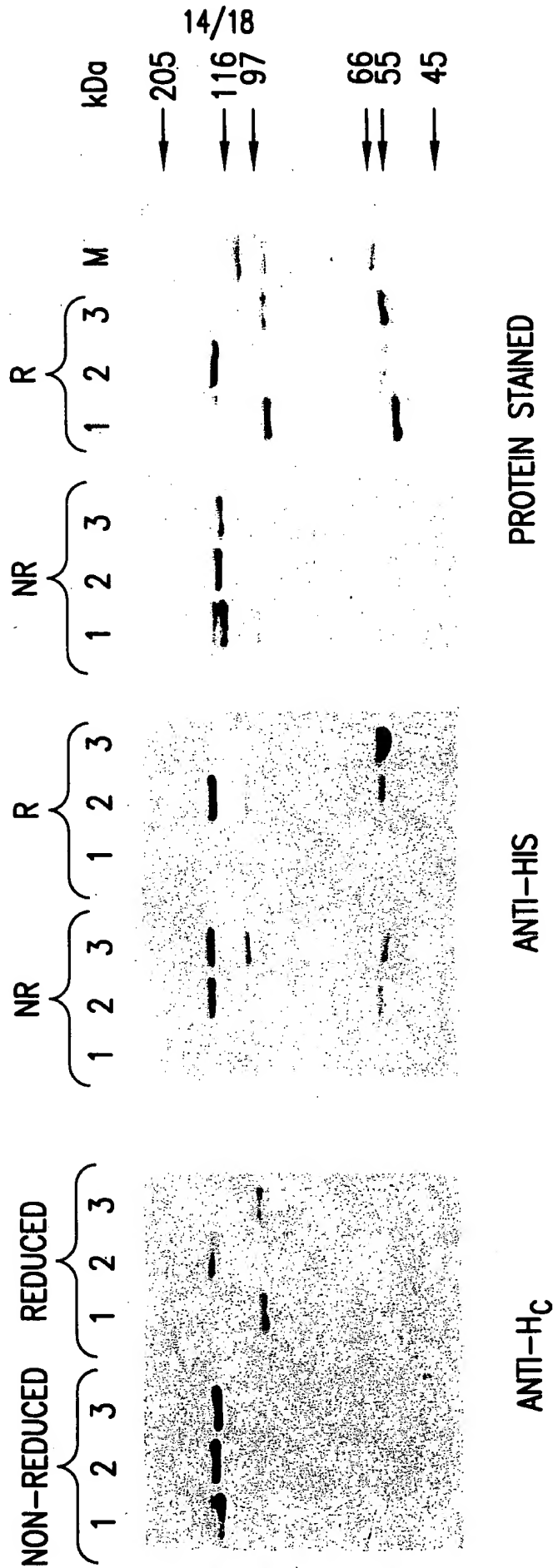
YIELD: 0.2mg/L OF
CULTURE

PROTEIN STAINED

WESTERN BLOTTED
WITH ANTI-HIS IgG

FIG.12

1: BoNT/E FROM *CLOSTRIDIUM BOTULINUM*
 2: UN-NICKED RECOMB. BoNT/E
 3: NICKED RECOMB. BoNT/E
 M: MARKERS



WESTERN BLOTTING

FIG.13

GST-SNAP-25(140-205)

INCUBATED WITH:

-DTT

+DTT

NATIVE DI-CHAIN

RECOMB. SINGLE-CHAIN

RECOMB. DI-CHAIN

(nM)

25

5

1

0.2

0.04

25

5

1

0.2

0.04

RECOMB. DI-CHAIN MUTANT

(nM)

0.2

1

5

25

125

0.2

1

5

25

125

FIG.14



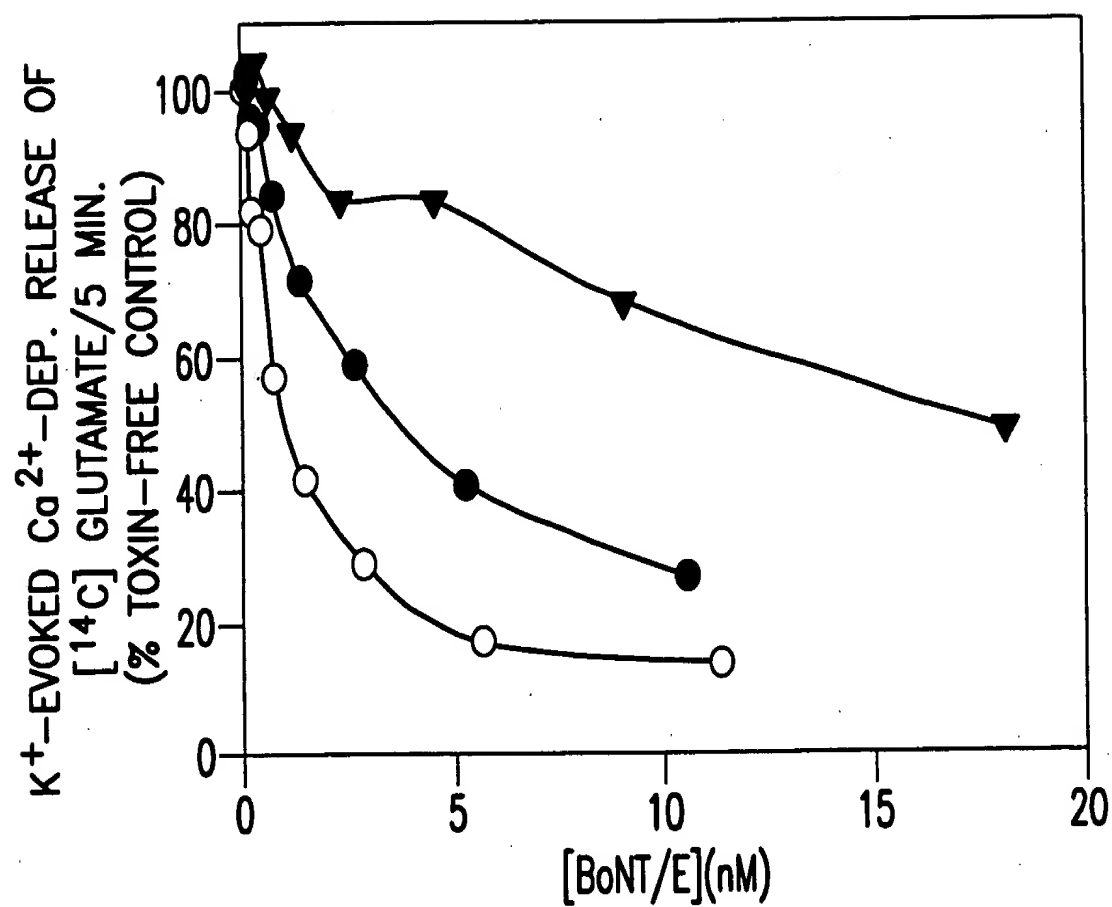


FIG. 15

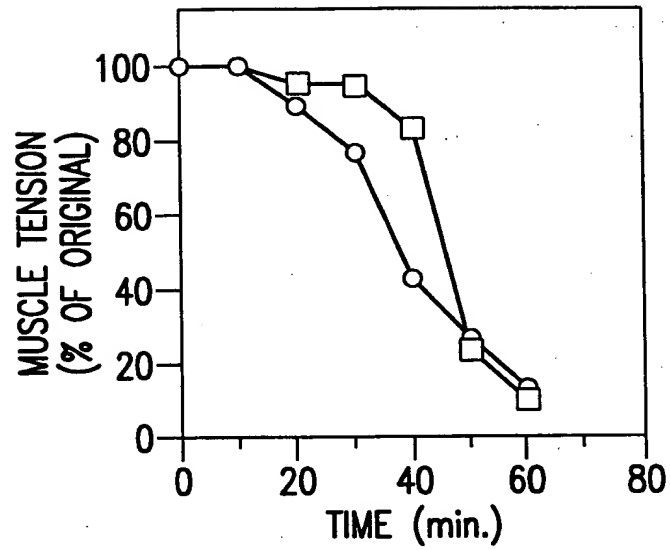


FIG. 16A

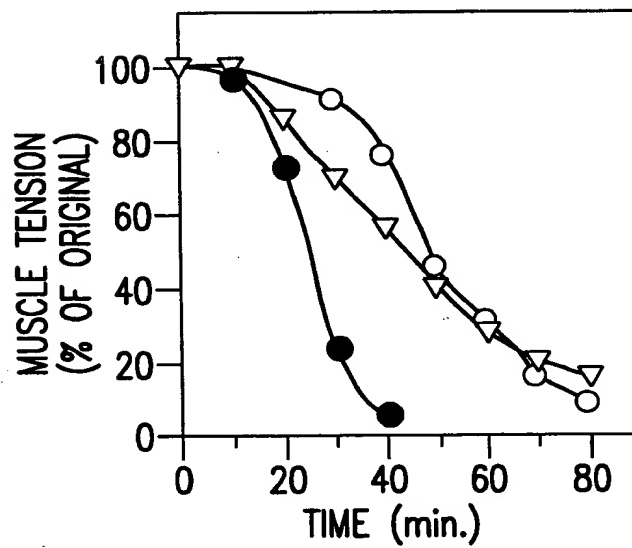


FIG. 16B

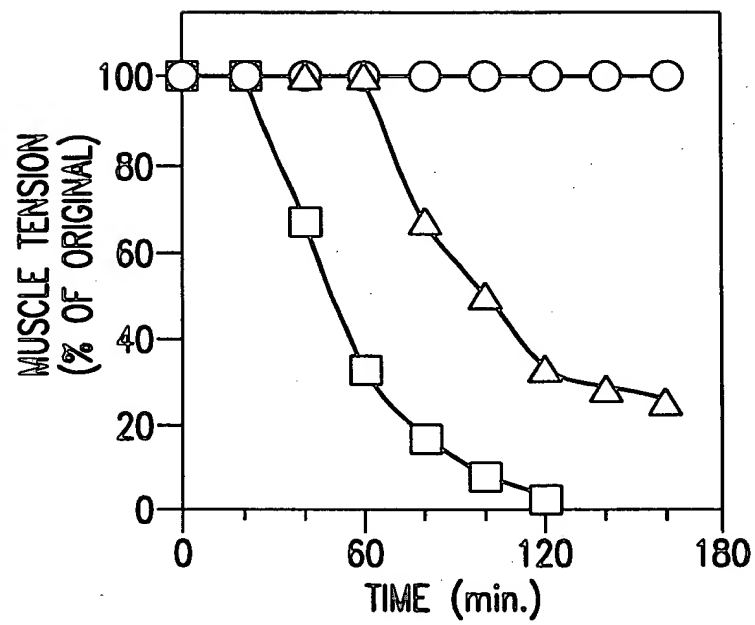


FIG. 17